*2017 International Nuclear Atlantic Conference - INAC 2017*
*Belo Horizonte, MG, Brazil, October 22-27, 2017*
*ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN*

Agência Brasileira do ISBN
ISBN 978-85-99141-07-6
9 788599 141076

# Characterization of ionizing radiation effects on bone using Fourier Transform Infrared Spectroscopy and multivariate analysis of spectra

**Pedro Arthur Augusto de Castro[1], Derly Augusto Dias[1] and Denise Maria Zezell[1]**

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
zezell@usp.br

## ABSTRACT

Ionizing radiation has been used as an important treatment and diagnostic method for several diseases. Optical techniques provides an efficient clinical diagnostic to support an accurate evaluation of the interaction of radiation with molecules. Fourier-transform infrared spectroscopy coupled with attenuated total reflectance (ATR-FTIR) is a label-free and nondestructive optical technique that can recognize functional groups in biological samples. In this work, 30 fragments of bone were collected from bovine femur diaphysis. Samples were cut and polished until 1 cm x 1 cm x 1 mm, which were then stored properly in the refrigerated environment. Samples irradiation was performed with a Cobalt-60 Gammacell Irradiator source at doses of 0.1 kGy, 1 kGy, whereas the fragments exposed to dose of 15 kGy was irradiated in a multipurpose irradiator of Cobalt-60. Spectral data was submitted to principal component analysis followed by linear discriminant analysis. Multivariate analysis was performed with Principal component analysis(PCA) followed by Linear Discriminant Analysis(LDA) using MATLAB R2015a software (The Mathworks Inc., Natick, MA, USA). We demonstrated the feasibility of using ATR-FTIR spectroscopy associated with PCA-LDA multivariate technique to evaluate the molecular changes in bone matrix caused by different doses: 0.1 kGy, 1 kGy and 15 kGy. These alterations between the groups are mainly reported in phosphate region. Our results open up new possibilities for protein monitoring relating to dose responses. This work was supported by CNPq/INCT 465763/2014-6, PQ 312397/2013-5 and CAPES/PROCAD 88881.068505/2014-01.

## 1. INTRODUCTION

Ionizing radiations, gamma or X-ray, demonstrate a broad range of applications such as medicine, industrial radiography, and food safety [1], [2], among others.

When bone is submitted to ionizing radiation, biochemical alterations can take place [3], [4], which may influence its mechanical properties.

The characterization of the irradiated bone tissue can be a tool in understanding which components are affected and how certain doses of ionizing radiation alter its molecular structure and mechanical properties. In the clinical diagnostic context, alternative techniques have been employed to promote an accurate bioanalysis.

Fourier-transform infrared spectroscopy coupled with attenuated total reflectance (ATR-FTIR) is an optical technique which reports variations of vibrational bonds and their fundamental feature in biological material. The ATR-FTIR can provide additional advantages in elucidating bone chemical compositional such as mineral-to-matrix ratios, carbonate-to-phosphate ratios, mineral crystallinity and collagen maturity.

The aim of this work is to characterize the bone chemical changes due to different radiation doses to improve the understanding of the role organic modification.

## 2. METHODS AND MATERIAL

### 2.1 Sample preparation

In this work, 30 fragments of bone were obtained from bovine femur diaphysis. All samples were cut into 1 cm x 1 cm x 1 mm, which were polished and stored in the refrigerated environment.

Irradiation of samples was performed with a Cobalt-60 Gammacell Irradiator source at doses of 0.1 kGy, 1 kGy, whereas the fragments exposed to a dose of 15 kGy was irradiated in a multipurpose irradiator of Cobalt-60, at IPEN.

### 2.2 ATR-FTIR Spectroscopy

Data acquisition. Conventional macro-ATR-FTIR measurements in the range of $4000 – 400\ cm^{-1}$ were recorded using a Smart Orbit ATR accessory coupled to a Thermo Nicolet 6700 FTIR system (Thermo Scientific, Waltham, MA, USA), equipped with a deuterated triglycine sulfate (DTGS) detector. Spectra were collected with spectral resolution of $4\ cm^{-1}$ and 100 scans per spectrum. Samples were pressed into ATR diamond crystal with standardized pressure and for each group, 100 scans were co-added, and the spectrum obtained represents the averaged from 10 measurements.

### 2.3 Multivariate Analysis

Raw ATR-FTIR spectra were truncated to a range of $1800\text{-}400\ cm^{-1}$, vector normalized before being converted to second derivatives and smoothed using Savitzky-Golay filter using a second order polynomial fit with an eleven points window. The implementation of multivariate analysis was carried out using the second derivative spectra. For representation of biochemical recognition, we applied Principal Component Analysis (PCA) followed by Linear Discriminant Analysis (LDA). All data were performed using homemade software in MATLAB R2015a (The Mathworks Inc., Natick, MA, USA).

## 3. RESULTS AND DISCUSSION

Figure 1 shows the vector normalized ATR-FTIR spectra, in which differences among the several irradiation doses received by the bone samples are not noticed.

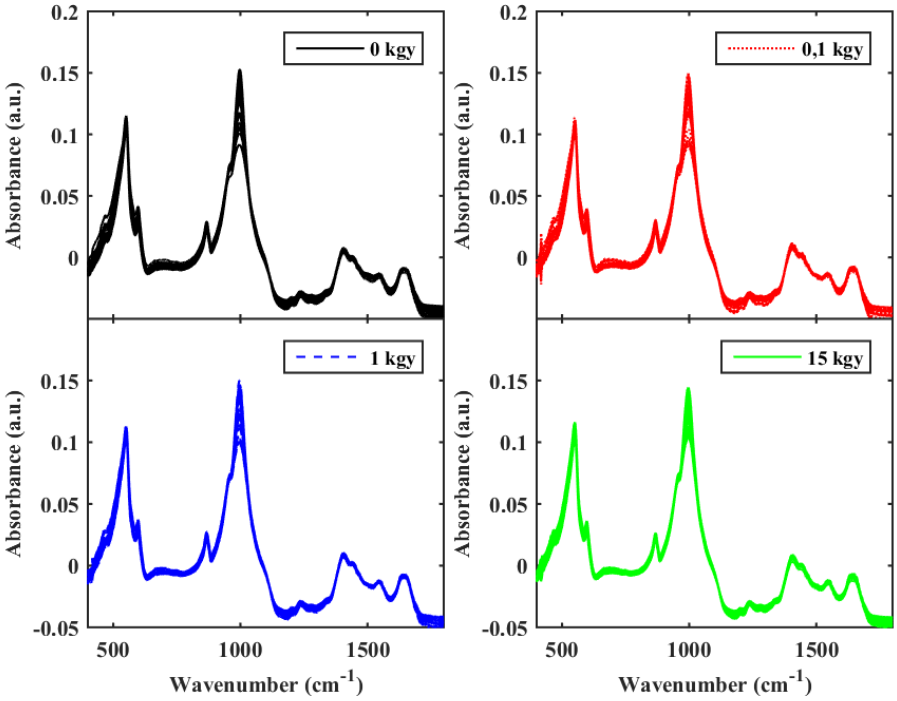


**Figure 1: Normalized ATR-FTIR spectra of bovine bone according to the dose received.**

The spectra from non-irradiated (0 kGy) are compared with other groups (0.1 kGy, 1 kGy and 15 kGy) to obtain the spectral differences. To enhance classification of the proper characteristics, which remark the dose-response effect, we use the second derivative averaged spectra of each dose group for the Principal component analysis (PCA) followed by Linear Discriminant Analysis (LDA).

PCA is a well-established chemometric methodology in which scores plots are used to identify the class-specific information of all observed class grouping [5]. In PCA, the loading plot gives the recognition of specific spectral peaks that are responsible for differences between groups.

LDA assigned a priori into classes for emphasizing the peculiarities of radiation effect on bone. In this way, linear discriminant variables are constructed (LD) focusing in the maximum separation of the clusters [6]. The LDs selected in Figure 2 showed the performance of non-irradiated and irradiated bone samples and correspondent spectral differences.

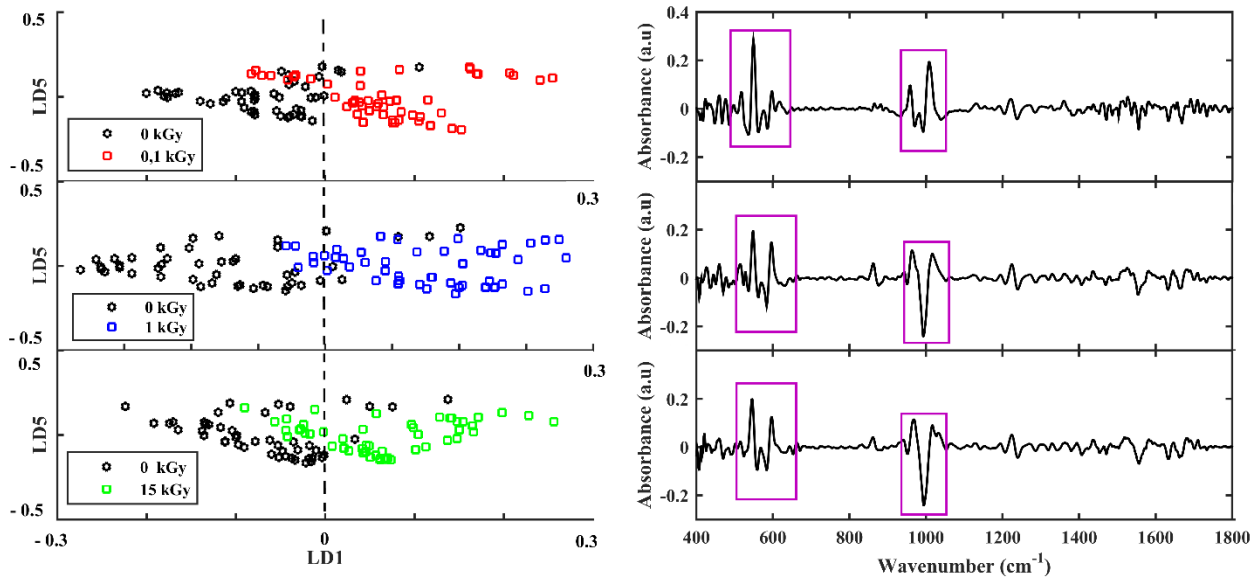


**Figure 2: Two-dimensional principal component analysis-linear discriminant analysis (PCA-LDA) scores plot (left side) and correspondent cluster vector plot (right side) with prominent phosphate region (highlighted in purple).**

Regarding potential modifications of the composition that will influence the molecular dynamics of material bonding, the most prominent spectral alterations are in the phosphate molecules, reported in Figure 2. These locations of absorption bands suggest modifications in mineralization maturity [7] in 0.1 kGy vs 0 kGy when it is matched with 1 kGy vs 0 kGy. The new bone formation can be expected in this phosphate region [8] and associated with the dose-effect.

The distribution of the spectral into clusters obtained with PCA-LDA scores plot, the accuracy, sensitivity, and specificity of classification was calculated as shown in Table 1.

**Table 1: Performance scores**

| Compared groups | Accuracy | Sensitivity | Specificity |
|---|---|---|---|
| 0 kGy vs 0.1 kGy | 88.77% | 93.88% | 83.67 % |
| 0 kGy vs 1 kGy | 87.75% | 87.76% | 87.76% |
| 0 kGy vs 15 kGy | 82.65 % | 89.8% | 75.51% |

Considering the accuracy results that were exhibited in Table 1, it is possible to recognize a decrease in the accuracy values proportional to the radiation dose.

## 4. CONCLUSIONS

We have presented the results of ATR-FTIR associated to PCA-LDA efficiency to recognize irradiated dose and identify the spectral prominence that is primarily responsible for differentiating the groups. The differences expressed between these dose-response categories can be thought of as corresponding to phosphate alterations. This could potentially allow visualization of biomarkers across the dose-response in bone material.


## ACKNOWLEDGMENTS

This work was supported by CNPq/INCT 465763/2014-6, PQ 312397/2013-5 and CAPES/PROCAD 88881.068505/2014-01.


## REFERENCES


1. C.J. Hernandez; T.M. Keaveny, "A biomechanical perspective on bone quality". *Bone,* **Volume 39**, pp.1173-1181 (2006).
2. Huyn Nguyen et al., "Sterilization of allograft bone: effects of gamma irradiation on allograft biology and biomechanics", *Cell Tissue Bank*, pp.93-105 (2006).
3. Lisa M. Miller et al., "In situ analysis of mineral content and crystallinity in bone using infrared micro-spectroscopy of the $\upsilon_4$ $PO_4^{3-}$ vibration"., *Biochimica et Biophysica Acta,***Volume 1527**, pp.11-19 (2001).
4. Richard Mendelsohn et al., "Infrared Spectroscopy, Microscopy, and Microscopic Imaing of Mineralizing Tissues: Spectra-Structure Correlations form Human Iliac Crest Biopsies"., *J.Biomed. Opt.*, **Volume 4**, pp.14-21(1999).
5. Matthew J Baker et al., "Using Fourier transform IR spectroscopy to analyze biological materials"., *Nature Protocol,* **Volume 9,** pp.1771-1791 (2014).
6. Francis L. Martin et al., "Identifying Variables Responsible for Clustering in Discriminant Analysis of Data from Infrared Microspectroscopy of Biological Sample"., *Journal of Computational Biology,* **Volume 14,** pp.1176-1184 (2007).
7. Lyudmila Spevak et al., "FTIRI Parameters describing Acid Phosphate Substitution in Biological Hydroxyapatite"., *Calcified Tissue International.,* **Volume 92**, pp.418-428 (2013).
8. Faibish D et al., "Infrared imaging of calcified tissue in bone biopsies from adults with osteomalacia"., *Bone.,* **Volume 36**, pp.6-12 (2005).